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(56) Documents Cited
GB 2296495 A GB 2157290 A US 4826964 A

(58) Field of Search
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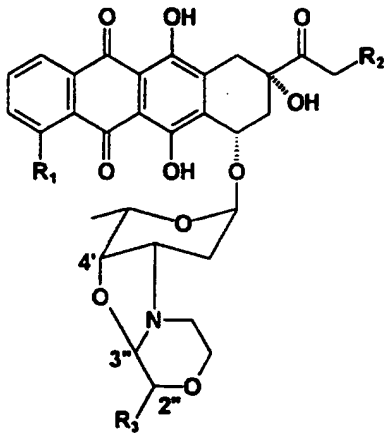
(54) Morpholinyl bridged anthracycline derivatives

(57) A morpholinyl derivative of formula 2



2

wherein: R_1 is methoxy, hydroxy or hydrogen, R_2 is hydrogen or hydroxy, R_3 is a C_1-C_5 alkoxy group, and the chiral carbon atom configuration at the 2" and 3" positions are 2"(S),3"(R),3"(S), or a pharmaceutically acceptable salt thereof.

The compounds are endowed with high antitumor activity.

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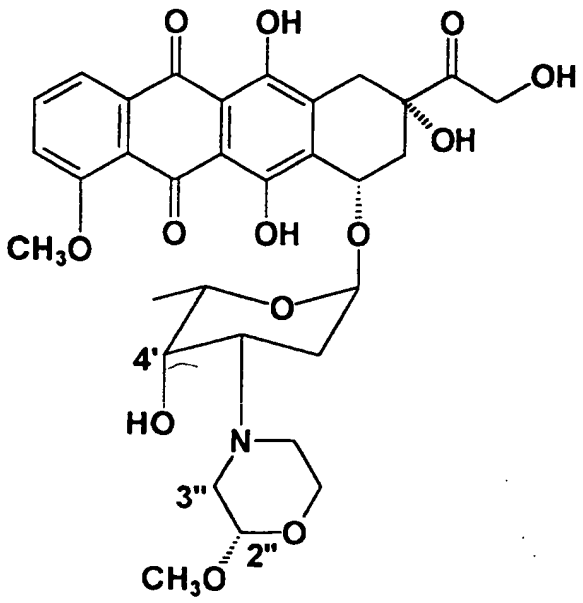
MORPHOLINYL ANTHRACYCLINE DERIVATIVES

The present invention relates to morpholinyl anthracycline derivatives, to a process for their preparation and to pharmaceutical compositions containing them.

The morpholinyl anthracyclines are semisynthetic analogs of anthracyclines endowed with remarkable antitumor activity (see: Bioactive Molecules Vol. 6, Ed. J.W. Lown, Elviser 1988). At present, several morpholinyl anthracyclines are under clinical evaluation. Among them, 3'-deamino-3'-[2"(S)-methoxy-4"-morpholinyl] doxorubicin (1) possesses high *in vitro* and *in vivo* activity on sensitive or multidrug resistant tumors including doxorubicin-resistant tumor cells (see: M. Grandi *et al.*, Cancer Treat. Rew., 17, 133, 1990 and M. Ripamonti *et al.*, Br. J. Cancer, 65, 703, 1992).



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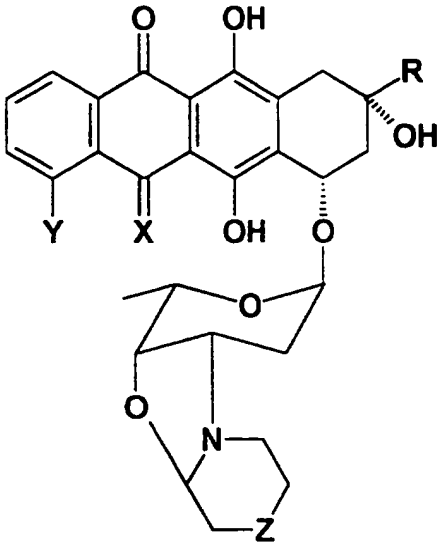
In US-A-4,826,964 (1989), E.D. Acton has described the preparation of bridged oxygen derivatives of daunorubicin and doxorubicin. US-A-4,826,964 also describes compounds of general formula A:



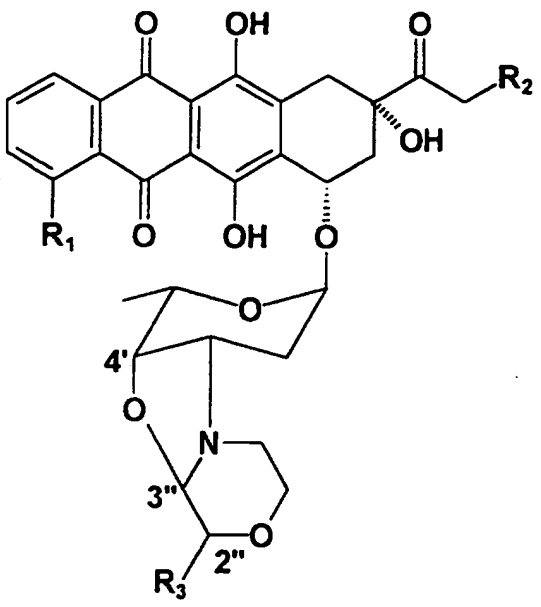
A

in which each of X and Z is an oxygen atom, Y is a methoxy group and R is the residue $-COCH_2OH$. Compounds of formula A were prepared in small amounts by reductive alkylation of anthracyclines with a dialdehyde of formula:

$$HOC-(CH_2)_2-O-(CH_2)_2-CHO$$

in the presence of reducing agents such as sodium or potassium cyanoborohydride.

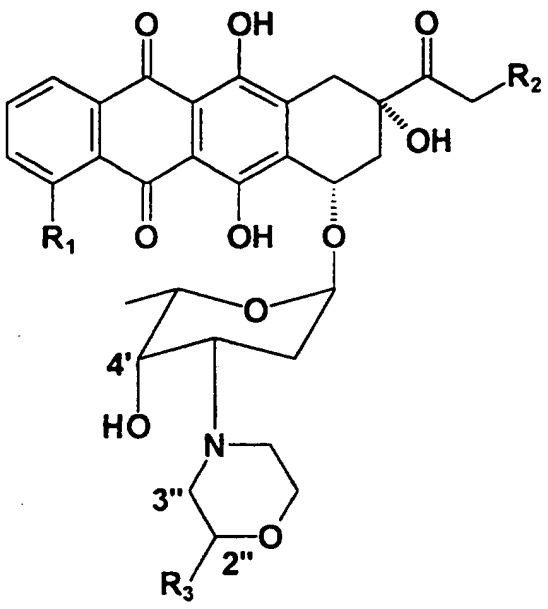
The present invention relates to new derivatives of morpholinyl anthracyclines in which the morpholino ring is bridged with an oxygen atom to the position C-4' of the sugar residue. The present invention provides a morpholinyl anthracycline derivative of formula 2:



2

wherein: R_1 is methoxy, hydroxy or hydrogen, R_2 is hydrogen or hydroxy, R_3 is a C_1-C_5 alkoxy group, and the chiral carbon atom configurations at the 2" and 3" positions are 2"(S),3"(R) or 2"(R),3"(S); or a pharmaceutically acceptable salt thereof. R^3 may be methoxy, ethoxy, propoxy, butoxy or pentoxy, preferably methoxy. R^1 is preferably methoxy. R^2 is preferably hydroxy. The invention also provides a process for the preparation of compounds of formula 2, pharmaceutical compositions containing them and the use thereof in treating certain mammalian diseases.

Compounds of formula (2), 3'-deamino-3",4'-anhydro-[2"-alkoxy-3"-hydroxy-4"-morpholinyl] anthracycline derivatives, show higher cytotoxic efficacy compared to that of the parent morpholinyl anthracyclines of formula 3:

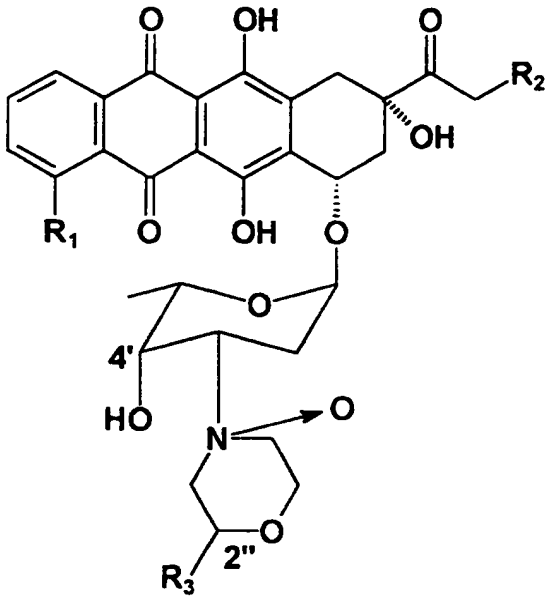


3

wherein R_1, R_2, R_3 and the configuration at C-2″ are as above defined.

The present invention also provides a process for preparing a compound of formula 2 or a pharmaceutically acceptable salt thereof, which comprises

(a) reacting an N-oxide anthracycline derivative of formula (4):



4

wherein R_1, R_2, R_3 and the configuration at C-2" are as above defined, with an iron (II) salt in presence of an iron-complexing agent, and

(b) if necessary converting the resultant compound of formula 2 into a pharmaceutically acceptable salt thereof.

It should be stressed that the presence of iron-complexing agent is crucial for the formation of compound 2. The reaction proceeds with formation of single isomer and formation of the parent morpholinyl anthracycline of formula 3, which may be recovered. Preferably, the iron salt is iron(II) chloride tetrahydate. Preferably, the complexing agent is tartaric acid. Preferably, the reaction is performed in water. The reaction can be carried out at room temperature. Typically, the reaction time is 48 hours.

The starting compounds of formula 4 may be prepared as described in GB-A-2296495.

The new anthracycline derivatives of the present invention are endowed with antitumoral activity.

The present invention also provides a pharmaceutical composition comprising a pharmaceutically acceptable diluent or carrier and, as an active principle, a morpholinyl anthracycline compound of formula 2 or a pharmaceutically acceptable salt thereof.

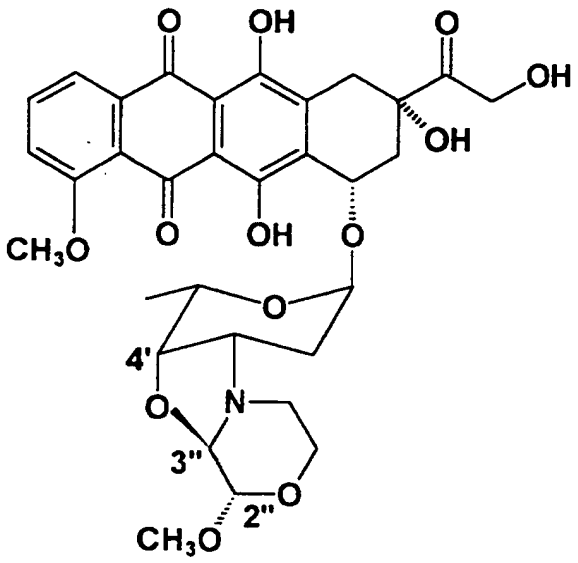
Suitable routes of administration include parenteral administration. For parenteral administration a liquid formulation may be prepared using the active compound and a sterile diluent or carrier which may either dissolve the active compound or provide a suspension for it. The parenteral formulation may be prepared in the form of a sterile solid for reconstitution prior to administration with a suitable vehicle such as physiological saline, sterile water or other sterile vehicle.

The compounds of the invention are useful in methods of treatment of leukaemia or colon adenocarcinoma. A therapeutically effective amount is administered to a patient having a tumor to ameliorate or improve the condition of the patient. An amount sufficient to inhibit the growth of the tumor may be administered. The dosage to be given can be ascertained using known dosage ranges for doxorubicin and daunorubicin modified by reference to the activity shown by the present compounds

in *in vitro* and *in vivo* anti-tumor tests. Suitable dosages are generally in the range of from 0.01 to 100 mg/m^2 body surface, preferably from 0.1 to 10 mg/m^2, depending on the nature and severity of the disease being treated and on the general condition of the patient.

The following Examples further illustrate the present invention.

Example 1: Preparation of 3'-deamino-3"-4'-anhydro-[2"(S)-methoxy-3"(R)-hydroxy-4"-morpholinyl]doxorubicin (2a)



2a

3'deamino-3'-[2"(S)-methoxy-4"-morpholinyl]-3'-N-oxide-doxorubicin (1, 0.16g, 0.234mmol) is dissolved in a water solution (10ml) containing iron(II) chloride tetrahydrate (0.11g) and tartaric acid (0.75g). The mixture is kept under stirring, in the dark at room temperature, for 48 hours. After that, methylene

chloride (50ml) is added and the crude product is extracted in the organic phase from which the title compound 2a is separated after flash chromatography on silica gel (eluting system: methylene chloride/ethanol 24/1 v/v). Yield 10 mg. TLC on kieselgel plate F_{254} (Merck), eluting system methylene chloride/methanol (9/1 v/v), R_f=0.62. FD-MS: E.H.C. 30mA; m/z 642 (93, [MH]+); m/z 641 (100, [M]+). The structure of compound 2a was assigned on the basis of ^{1}HNMR studies.

^{1}HNMR (400 MHz, $CDCl_3$) δ:

1.38 (d, J=6.4Hz, 3H, $\underline{CH_3}$-5'); 1.72 (ddd, J=6.2, 6.2, 15.4Hz, 1H, H-2'β); 2.01 (ddd, J=5.5, 5.5, 15.4Hz, 1H, H-2'α); 2.10 (dd, J=3.8, 14.9Hz, 1H, H-8az); 2.49 (ddd, J=1.7, 2.1, 14.9Hz, 1H, H-8eq); 2.73 (m, 1H, H-4"ax); 2.76 (m, 1H, H-2"eq); 3.00 (t, J=3.8Hz, 1H, CH_2-$\underline{OH}$); 3.05 (d, J=19.2Hz, 1H, H-10ax); 3.25 (dd, J=1.7, 19.2Hz, 1H, H-10eq); 3.37 (m, 1H, H-3'); 3.45 (s, 3H, $\underline{OCH_3}$-2"); 3.58 (m, 1H, H-5"eq); 3.91 (m, 1H, H-5"ax); 3.99 (dq, J=1.7, 6.4Hz, 1H, H-5'); 4.08 (dd, J=1.7, 6.4Hz, 1H, H-4'); 4.08 (s, 3H, $\underline{OCH_3}$-4); 4.45 (d, J=2.1Hz, 1H, H-3"); 4.69 (d, J=2.1Hz, 1H, H-2"); 4.75 (d, J=3.8Hz, 2H, $\underline{CH_2}$OH); 4.87 (s, 1H, OH-9); 5.31 (dd, J=2.1, 3.8Hz, 1H, H-7); 5.47 (dd, J=5.5, 6.2Hz, 1H, H-1'); 7.39 (d, J=7.7Hz, 1H, H-3); 7.78 (dd, J=7.3, 7.7Hz, H-2); 8.02 (d, J=7.3Hz, 1H, H-1); 13.25 (s, 1H, OH-11); 13.99 (s, 1H, OH-6).

Example 2: Biological Activity

3'-Deamino-3",4'-anhydro-[2"(S)-methoxy-3"(R)-hydroxy-4"-morpholinyl]doxorubicin 2a was tested *in vitro* on L1210 leukemia cells in comparison with 3'-deamino-3'-[2"(S)-methoxy-4"-morpholinyl]doxorubicin (1). The cytotoxic activity is reported as IC_{50}, the concentration inhibiting 50% of colony growth, calculated on concentration response curves. Compound 2a was found 2.5×10^3 fold more potent than compound 1 (Table 1).

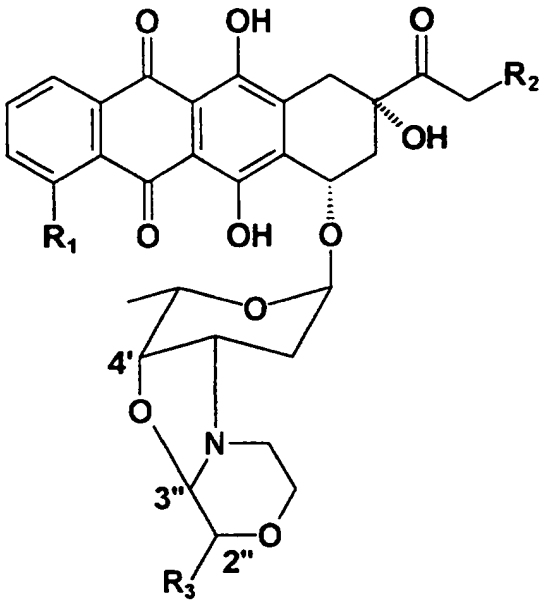
Table 1: *In vitro* cytotoxic activity (IC_{50}) of 3'-deamino-3",4'-anhydro-[2"(S)-methoxy-3"-hydroxy-4"-morpholinyl]doxorubicin (2a) and 3'-deamino-3'-[2"(S)-methoxy-4"-morpholinyl]doxorubicin (1) on L1210 leukemia cells.

Growth inhibition test: 48h treatment

Compound	IC_{50}
2a	0.000292 ± 0.00008
1	7.38 ± 1

CLAIMS

1. A morpholinyl derivative of formula 2:



2

wherein: R_1 is methoxy, hydroxy or hydrogen, R_2 is hydrogen or hydroxy, R_3 is a C_1-C_5 alkoxy group, and the chiral carbon atom configurations at the 2" and 3" positions are 2"(S), 3"(R) or 2"(R), 3"(S); or a pharmaceutically acceptable salt thereof.

2. A compound according to claim 1 wherein R_2 is hydroxy.

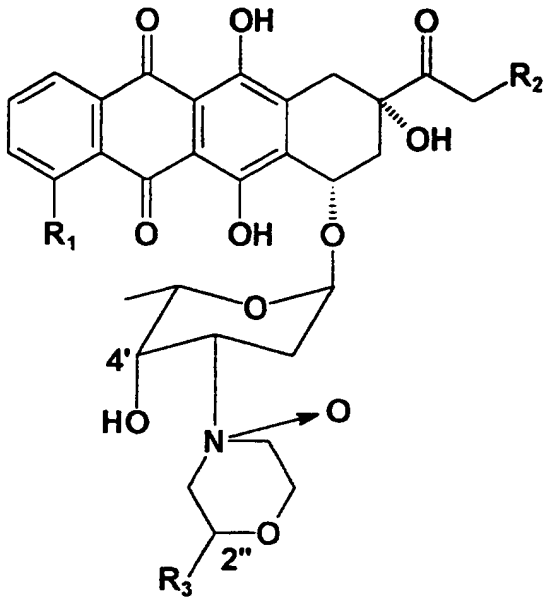
3. A compound according to claim 1 or 2 wherein R_1 is methoxy.

4. A compound according to any one of the preceding claims wherein R_3 is methoxy.

5. A compound according to any one of claims 1 to 4 which is 3'-deamino-3",4'-anhydro-[2"(S)-methoxy-3"(R)-hydroxy-4"-morpholinyl] doxorubicin.

6. A process for the preparation of a compound as defined in claim 1, which comprises

(a) reacting a N-oxide anthraycline derivative of formula 4:



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wherein R_1, R_2, R_3 and the configuration at C-2" are as defined in claim 1, with an iron(II) salt in presence of an iron complexing agent, and

(b) if necessary converting the resultant morpholinyl derivative of formula 2 into a pharmaceutically acceptable salt thereof.

7. A process according to claim 6, wherein step (a) is carried out in water, the iron salt is iron(II) chloride tetrahydrate and the complexing agent is tartaric acid.

8. A process for preparing a compound as defined in claim 1, which process is substantially as described in Example 1.

9. A pharmaceutical composition comprising a pharmaceutically acceptable diluent or carrier and, as an active principle, a morpholinyl derivative of formula (2), or a pharmaceutically acceptable salt thereof, as defined in any one of claims 1 to 5.

10. A morpholinyl derivative of formula 2, or a pharmaceutically acceptable salt thereof, as defined in any one of claims 1 to 5 for use in a method of treatment of the human or animal body by therapy.

11. A morpholinyl derivative of formula 2, or a pharmaceutically acceptable salt thereof, as defined in any one of claims 1 to 5 for use as an anti-tumor agent.

12. Use of a morpholinyl derivative of formula 2, or a pharmaceutically acceptable salt thereof, as defined in any one of claims 1 to 5 in the manufacture of a medicament for the treatment of a tumor.

The Patent Office

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Application No: GB 9614621.2 **Examiner:** William Thomson
Claims searched: 1-12 **Date of search:** 15 July 1997

Patents Act 1977
Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.O): C2C (CUA)

Int Cl (Ed.6): C07H 15/252

Other: ONLINE: WPI, EDOC, CAS-ONLINE

Documents considered to be relevant:

Category	Identity of document and relevant passage		Relevant to claims
A	GB 2296495A	(PHARMACIA SPA) See whole document, in particular page 3, lines 1-4 and claims 1 and 6	
A	GB 2157290A	(SRI INTERNATIONAL) See whole document, in particular page 1, lines 42-44 and 54-56	
A	US 4826964	(SRI INTERNATIONAL) See whole document, in particular column 2, line 2 - column 3, line 4	

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category	P	Document published on or after the declared priority date but before the filing date of this invention